Human Vaccines & Immunotherapeutics 9:2, 314–321; February 2013; © 2013 Landes Bioscience

Detection of systemic and mucosal HPV-specific IgG and IgA antibodies in adolescent girls one and two years after HPV vaccination

Mirte Scherpenisse,[1,2,*] Madelief Mollers,[2,3] Rutger M. Schepp,[1] Chris J.L.M. Meijer,[2] Hester E. de Melker,[3] Guy A.M. Berbers[1] and Fiona R.M. van der Klis[1]

[1]Laboratory for Infectious Diseases and Screening; National Institute of Public Health and the Environment; Bilthoven, the Netherlands; [2]Department of Pathology; VU University Medical Centre; Amsterdam, the Netherlands [3]Department of Epidemiology and Surveillance; National Institute of Public Health and the Environment; Bilthoven, the Netherlands;

Keywords: HPV, cervical secretion, antibody concentrations, multiplex-immunoassay, transudation, exudation, HPV vaccination, IgG, IgA

Abbreviations: CIN, cervical intraepithelial neoplasia; CVS, cervical secretion samples; GMCs, geometric mean concentrations; HAVANA, HPV amongst vaccinated and non-vaccinated adolescents; 95% CI, 95% confidence interval; LU/ml, Luminex units/milliliter; Tt, tetanus toxoid; Dt, diphtheria toxoid; M, month (s); r_s, spearman rank correlation coefficient

The bivalent HPV16/18 vaccine induces high antibody concentrations in serum while data about antibody responses in the cervix are limited. In this study, we investigated pre- and post-vaccination antibody responses against seven high-risk HPV types by detection of IgG and IgA HPV-specific antibodies in cervical secretion samples (CVS) and serum. From an HPV vaccine monitoring study CVS and serum samples were available (pre-vaccination (n = 297), one year (n = 211) and two years (n = 141) post-dose-one vaccination) from girls aged 14–16 y. The girls were vaccinated with the bivalent HPV vaccine at months 0, 1 and 6. CVS was self-sampled using a tampon. Samples were tested for HPV-specific antibodies (HPV16/18/31/33/45/52/58) by a VLP-based multiplex immunoassay. Post-vaccination, IgG and IgA antibody levels for HPV16/18 were detectable in CVS and amounted to 2% and 1% of the IgG and IgA antibody levels observed in serum, respectively. The antibody levels remained constant between one and two years after vaccination. The correlation between CVS and serum was similar for IgG and IgA vaccine-derived antibody levels for HPV16 ($r_s = 0.58$, $r_s = 0.54$) and HPV18 ($r_s = 0.50$, $r_s = 0.55$). Vaccine-derived IgG antibody levels against cross-reactive HPV types in CVS and in serum were highest for HPV45. No IgA cross-reactive antibody responses could be detected in CVS. Post-vaccination, HPV16/18 IgG and IgA antibodies are not only detectable in serum but also in CVS. The correlation of HPV16/18 IgG antibody levels between serum and CVS suggests that vaccine induced HPV antibodies transudate and/or exudate from the systemic circulation to the cervical mucosa to provide protection against HPV infections.

Introduction

The HPVs that cause ano-genital cancers are sexually transmitted and can infect the basal cells of the cervical epithelium. Therefore, HPV vaccines need to induce protective antibody levels at the cervix where HPV-specific antibodies can prevent infection of keratinocytes.[1,2] Prophylactic vaccination with the two available HPV vaccines, a bivalent and a quadrivalent protects against infections with the most common high-risk HPV types detected in HPV associated cancers, HPV16 and 18. Both vaccines have proven to be very efficacious in the prevention of cervical intraepithelial neoplasia (CIN) in HPV naïve women.[3,4] Also protection against CIN2+ of cross-reactive HPV types has been observed and for the bivalent vaccine this amounted to 84%, 59% and 50% for HPV31, 33 and 45 up to 4 y after vaccination, respectively.[5,6] Antibody levels were found to be 10–100 times higher in vaccinated individuals as compared with naturally infected individuals,[7] while the mechanism by which vaccine-induced antibodies contribute to antibody levels at the cervix is not yet completely understood. Vaccine-induced antibodies localized in the genital tract might be derived from the systemic circulation by transudation or exudation of antibodies across the cervical epithelium to the mucus as a result of micro-lesions of the cervical epithelium that can easily occur e.g., during sexual intercourse.[8,9]

In the Netherlands, the bivalent HPV vaccine was included in the national immunization program in 2010 for girls 12 y of age. A catch-up vaccination campaign was performed for girls 13–16 y of age.[10]

Here, we present data of IgG and IgA HPV-specific antibody levels pre- and up to two years post-vaccination in self-sampled

*Correspondence to: Mirte Scherpenisse; Email: Mirte.Scherpenisse@rivm.nl
Submitted: 09/04/12; Revised: 10/22/12; Accepted: 10/28/12
http://dx.doi.org/10.4161/hv.22693

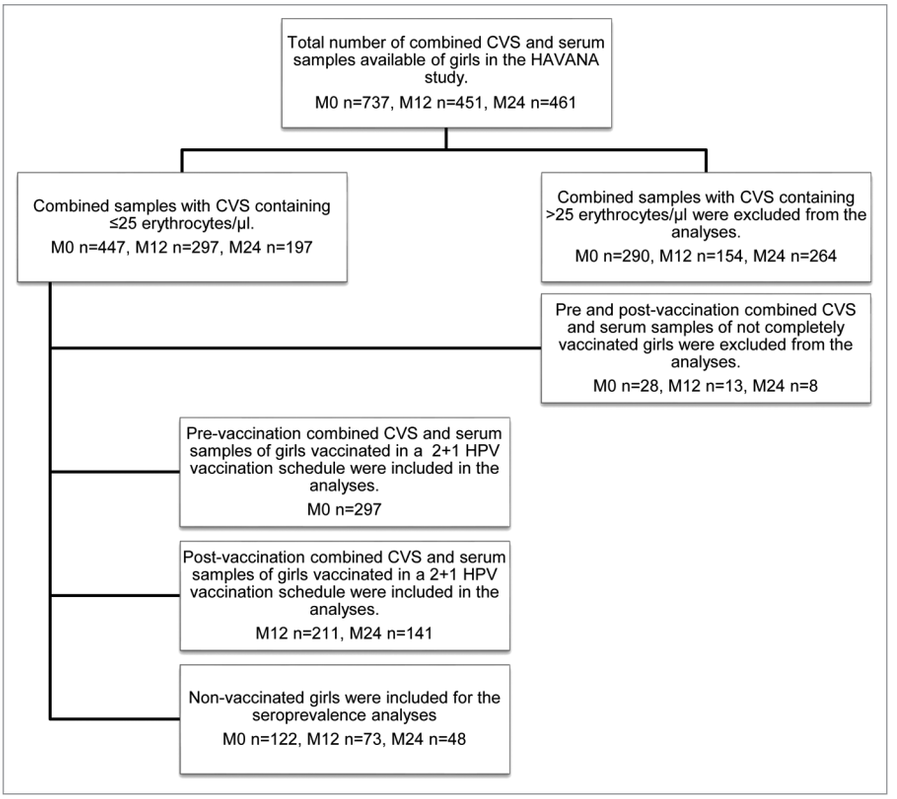

Figure 1. Flow diagram of available cervical secretion samples (CVS) and serum samples.

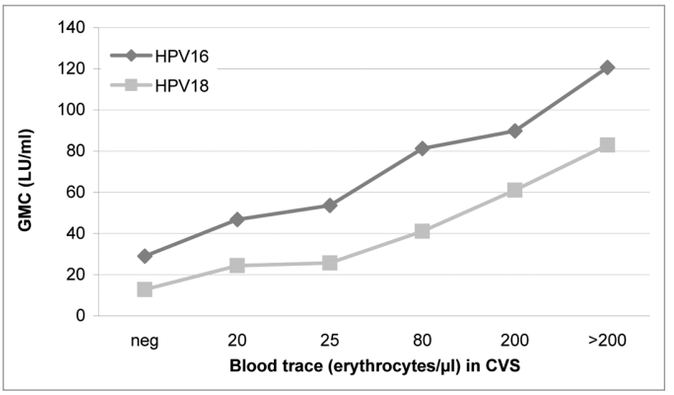

Figure 2. The effect of blood contamination on HPV16 (dark gray line) and HPV18 (light gray line) geometric mean concentrations (GMC) in cervical secretion samples (CVS).

cervical secretion and serum samples for HPV types 16, 18, 31, 33, 45, 52 and 58 of adolescent girls eligible for catch-up vaccination.

Results

Study characteristics. The mean age of the participating girls at the beginning of the study was 15.1 y. Girls were vaccinated with the bivalent HPV vaccine in a 2+1 vaccination schedule at months (M) 0, M1 and M6. At baseline (M0) 297 out of 737 girls provided both a cervical secretion sample (CVS) containing a blood trace of ≤ 25 erythrocytes/µl and serum sample (**Fig. 1**). One year (M12) and 2 y after the first vaccination (M24) 211/451 and 141/461 of these combined samples were available, respectively. For the non-vaccinated girls at M0 (n = 122), M12 (n = 73) and at M24 (n = 48) the combination of a CVS containing a blood trace of ≤ 25 erythrocytes/µl and serum sample was available. The use of oral contraceptives (OC) in vaccinated girls increased from 29% (87/297) at M0 up to 53% (111/211) at M12.

Evaluation of the measurement of HPV-specific antibodies in CVS collected with tampons. The tampon self-collection method was evaluated by measuring the recovery of HPV16 IgG and IgA antibody levels in CVS before and after tampon extractions. CVS (n = 25) were pooled and spiked with a sample of HPV16 IgG and IgA with antibody concentrations varying from low to high antibody levels. Importantly, the concentrations of HPV16 IgG and IgA antibodies before and after the tampon extractions were similar although not all the CVS volume can be centrifuged from the tampon. This spiking experiment indicates that there is no effect of the tampon on the HPV-specific antibody concentration in CVS.

As high antibody levels are present in serum even small blood traces could account for a considerable contribution to the antibody levels in CVS. We found that HPV16 and 18 antibody concentrations in CVS with a blood trace of ≤ 25 erythrocytes/µl were comparable to antibody concentrations in CVS with no blood trace (**Fig. 2**). However, in CVS with blood traces ≥ 25 erythrocytes/µl we observed an increasing linear trend in HPV16 (p = 0.02) and HPV18 (p = 0.03) antibody concentrations.

The effect of OC use on vaccine-derived HPV16 and 18 antibody levels one year after the first HPV vaccination were not significantly different between OC users and non-OC users (data not shown).

IgG and IgA antibody concentrations for HPV16 and 18 and phylogenetically related HPV types in serum. Pre-vaccination, only 10/297 (3.4%, 95% CI 1.7–5.9%) and 9/297 (3.0%, 95% CI 1.5–5.5%) girls showed IgG antibody concentrations in their serum above the cut-off values for HPV16 and HPV18, respectively. HPV16 and HPV18 geometric mean concentrations (GMCs) (0.5 and 0.6 LU/ml, respectively) were far below cut-off values. Post-vaccination (6 mo after the last vaccine dose), all girls seroconverted. HPV16 and 18 antibody concentrations significantly increased as compared with pre-vaccination and GMCs amounted to 3162 LU/ml (95% CI, 2712–3686 LU/ml) and 1611 LU/ml (95% CI, 1357–1913 LU/ml),

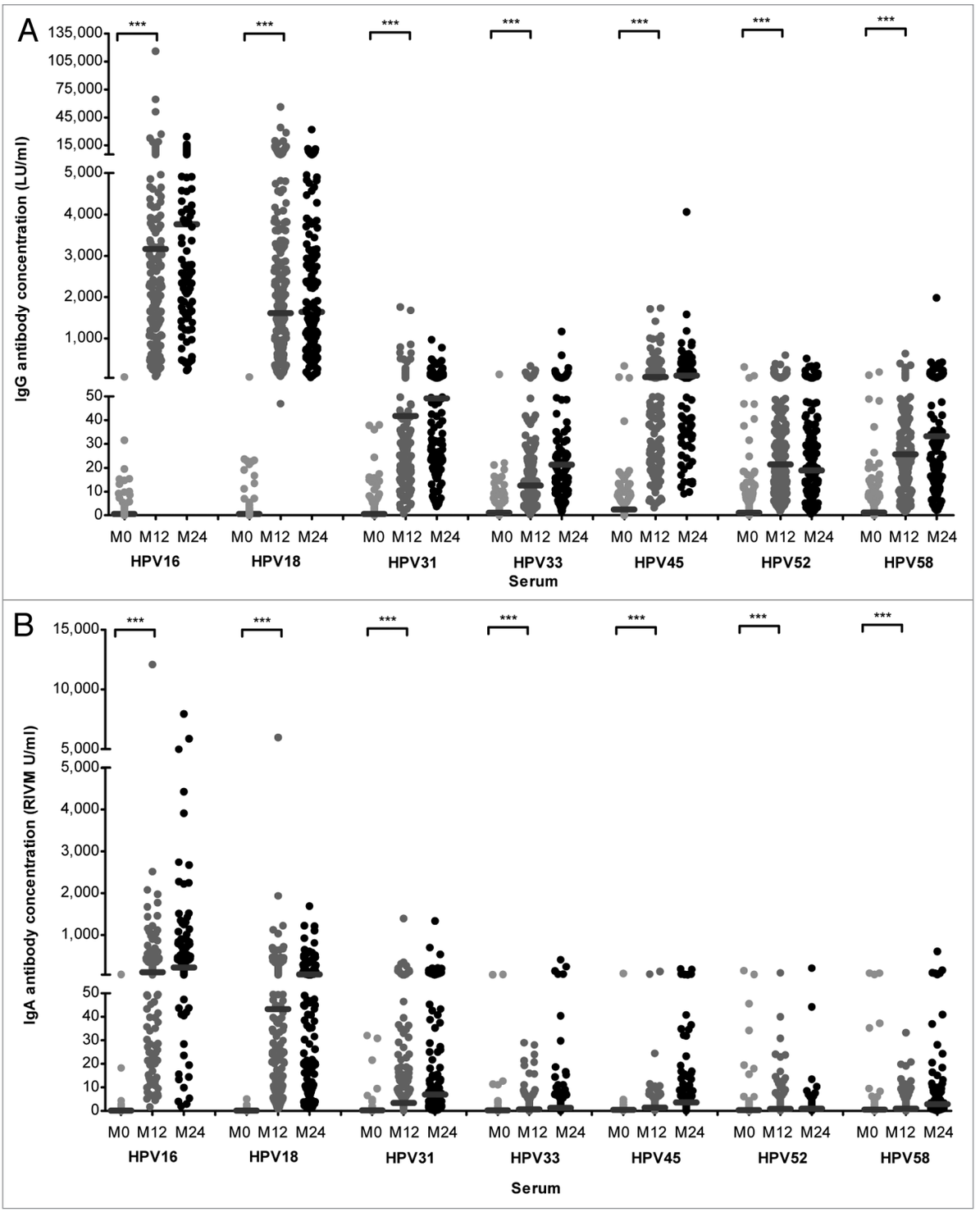

Figure 3. Antibody responses for HPV16, HPV18 and phylogenetically related HPV types 31, 33, 45, 52 and 58 in serum for IgG (**A**) and IgA (**B**) pre-vaccination (M0, light gray dots), one year after the first vaccination (M12, dark gray dots) and two years after the first vaccination (M24, black dots). ***p < 0.0001.

respectively (**Fig. 3A**). These HPV antibody concentrations remained constant in the subsequent year, thus 6–18 mo after the last vaccination. A similar result (no decrease) was found when the analysis was restricted to girls who provided samples at both 6 and 18 mo (n = 69 pairs) after the last vaccine dose.

Post-vaccination cross-reactive IgG antibody responses were highest for HPV45 and from all girls tested, 89% (95% CI 84–93%) showed antibody concentrations above the cut-off values for HPV45. For HPV31, 33, 52 and 58 this resulted in 66% (95% CI 59–72%), 55% (95% CI 48–61%), 62%

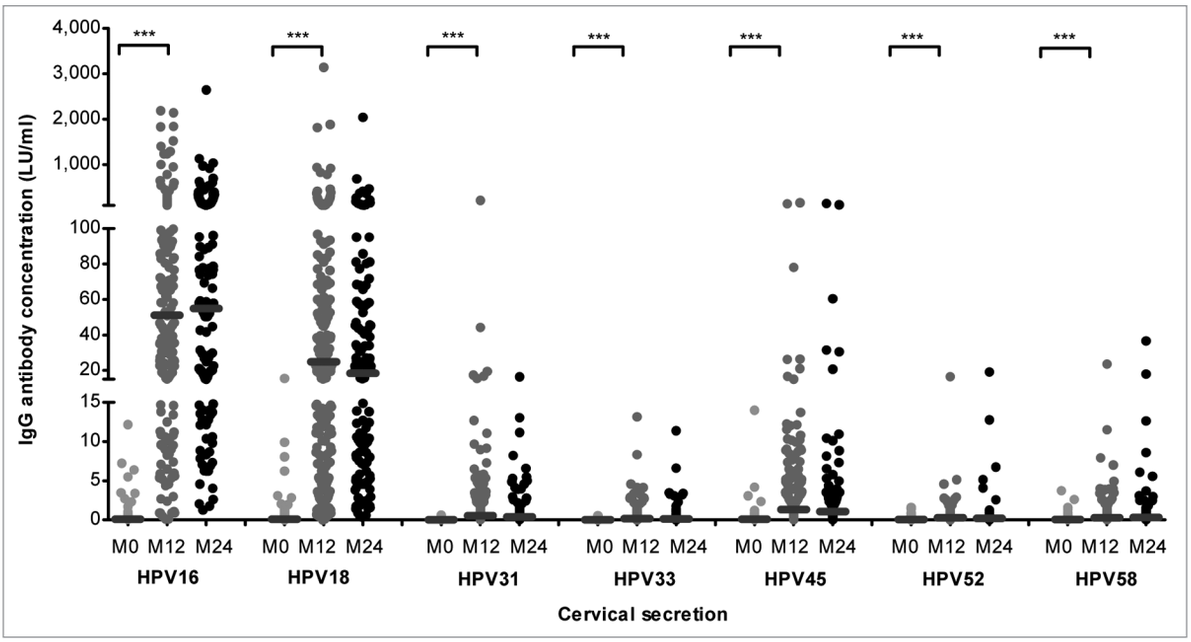

Figure 4. IgG antibody responses for HPV16, HPV18 and for phylogenetically related HPV types 31, 33, 45, 52 and 58, pre-vaccination (M0, light gray dots), one year after the first vaccination (M12, dark gray dots), and two years after the first vaccination (M24, black dots) in cervical secretion. ***p < 0.0001.

(95% CI 55–68%) and 43% (95% CI 37–50%) seropositivity, respectively. Cross-reactive HPV antibody concentrations remained constant in the subsequent year.

Pre-vaccination, HPV16 and HPV18 IgA GMCs amounted to 0.8 and 0.1 RIVM U/ml, respectively. After vaccination, HPV16 and HPV18 IgA antibody responses in serum at M12 rose to 112 RIVM U/ml (95% CI 93–136 RIVM U/ml) and 43 RIVM U/ml (95% CI 35–54 RIVM U/ml), respectively, and similar antibody levels were found at M24 (**Fig. 3B**). The cross-reactive IgA antibody levels for the other 5 HPV types increased after vaccination which was most pronounced for HPV31 but for the other cross-reactive HPV types this increase was quite low.

IgG and IgA antibody concentrations for HPV16 and HPV18 and phylogenetically related HPV types in cervical secretion. Before vaccination in CVS, HPV16 and 18 GMCs were near detection limits (0.1 LU/ml) and significantly increased after vaccination. IgG antibody concentrations detected in CVS amounted to approximately 2% of the IgG serum antibody concentrations both for HPV16 (GMC 51 LU/ml, 95% CI 41–64 LU/ml) and 18 (GMC 25 LU/ml, 95% CI 20–31 LU/ml) (**Fig. 4**). At M12 88% (95% CI 83–92%) and 70% (95% CI 63–76%) of the girls had IgG antibody concentrations in CVS above cut-off values for HPV16 and HPV18, respectively. These levels remained constant up to M24.

The 5 cross-reactive HPV types also showed a significant increase in IgG antibody concentrations at M12 compared with M0, however, few girls had antibody levels above cut-off values varying from 0–3% for HPV58 to HPV45. Similar to serum antibody levels, highest cross-reactive antibody levels in CVS were also found for HPV45. Cross-reactive HPV antibody concentrations in CVS remained constant up to M24.

The IgA antibody concentrations for HPV16 and 18 in CVS amounted to approximately 1% of serum IgA antibody levels. For the other 5 HPV types no IgA responses could be detected.

Seropositivity in non-vaccinees. Non-vaccinated girls were tested for HPV antibody seropositivity at M0 (n = 122), M12 (n = 73) and M24 (n = 48) (**Fig. 1**). In non-vaccinated girls, a higher HPV16 IgG seroprevalence was found at M12 (5/73 girls, 6.8%, 95% CI 2.6–14.5%) and M24 (6/48 girls, 12.5%, 95% CI 5.2–24.2%) compared with M0 (2/122 girls, 1.6%, 95% CI 0.3–5.3%). At M12, GMCs of HPV seropositive girls were approximately 150 times and 40 times lower as compared with vaccine induced antibody concentrations for HPV16 and 18, respectively. For HPV45 and 33 similar seroprevalences were found as compared with HPV16. For the other 4 HPV types a small rise in seroprevalence was observed at M24: 2/48 girls for HPV18 and HPV31 as compared with M0 (0/122) and 1/48 girls for HPV52 and HPV58 as compared with M0 (1/122). In CVS, IgG antibodies above cut-off values were only detectable for HPV16 and 18 at M12 (HPV16/18 both in 1/73 girls) and at M24 (HPV16 2/48 girls, HPV18 1/48 girls). IgA antibodies in CVS and serum samples were very low and near the detection limit.

Correlations between serum and cervical secretion IgG and IgA antibody levels. One year after vaccination we found similar correlations between the IgG antibody concentrations in serum and CVS for both HPV16 (r_s = 0.58) and HPV18 (r_s = 0.50) (**Fig. 5A and B**, respectively). Although IgA antibody levels in CVS were low also similar correlations between serum and CVS IgA levels were found for HPV16 (r_s = 0.54) and HPV18 (r_s = 0.55) (**Fig. 5C and D**, respectively).

Despite the lower levels in CVS, we observed after vaccination at M12 a high correlation between the HPV16 and HPV18 IgG

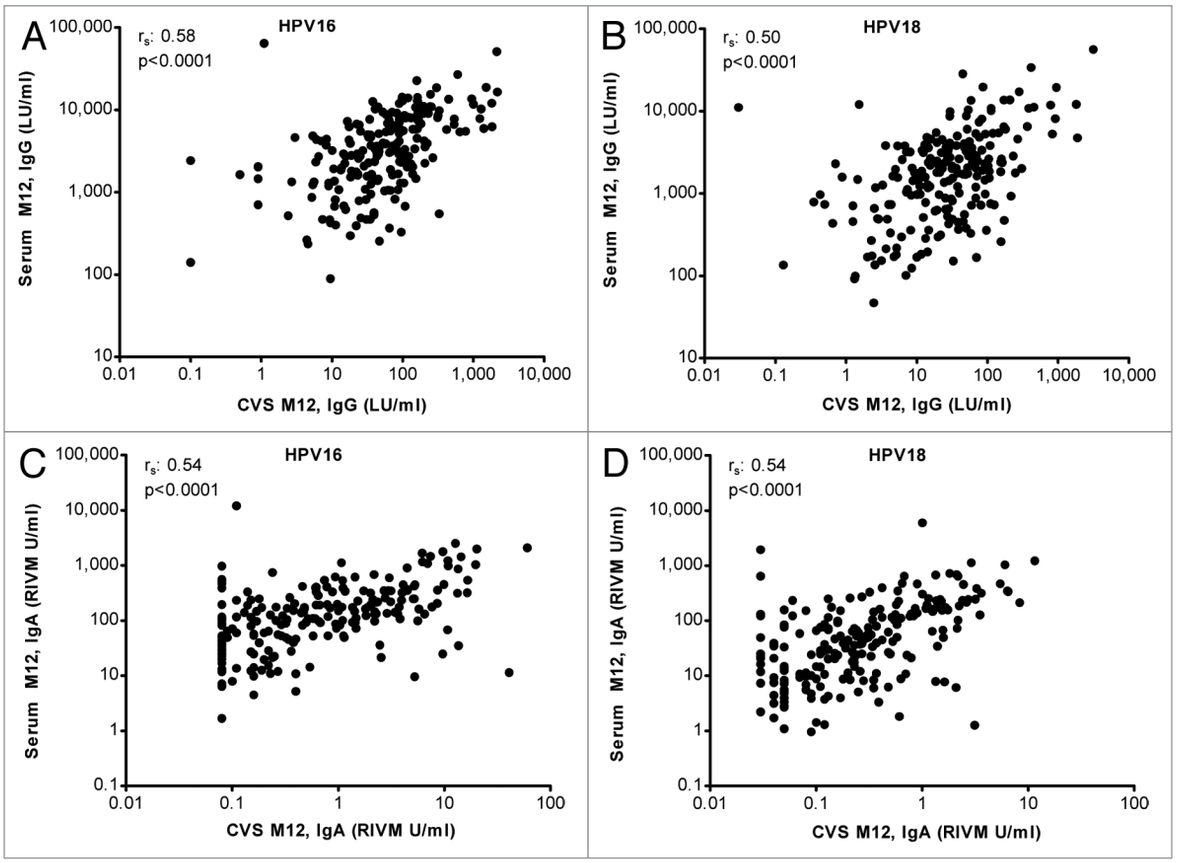

Figure 5. Spearman rank correlations (r_s) between HPV16 and 18 IgG (**A and B**) and IgA antibody levels (**C and D**) in serum and cervical secretion samples (CVS) one year after the first vaccination (M12). X and Y-axis are in logarithmic scale.

antibody levels in serum (r_s = 0.83) and CVS (r_s = 0.88) (**Fig. 6A and B**, respectively). Comparable correlations were found at M24. Normalizing the antibody concentrations in CVS and in serum to the amount of total IgG, we did not observe any alterations in the correlations for both HPV16 and HPV18.

IgG antibodies against tetanus and diphtheria toxoid in cervical secretion and serum. We determined the presence of vaccine induced IgG antibody levels against tetanus (Tt) and diphtheria toxoid (Dt) in serum and CVS. The cervical mucosa is not a site of local production of antibodies for these pathogens and therefore the detection of Dt and Tt specific antibodies in CVS can be used as a transudation or exudation marker. Interestingly, we found a high correlation between IgG antibody concentrations in serum and in CVS for Tt (r_s = 0.73, n = 35) and Dt toxoid (r_s = 0.78, n = 35) in samples of girls at M12 (data not shown).

Discussion

In this study, we could establish the presence of HPV-specific IgG and IgA antibodies in CVS but the levels were much lower than in serum. Constant HPV16/18 IgG antibody levels in serum and CVS were found up to two years post-vaccination.

HPV16/18 IgG and IgA levels in CVS amounted to approximately 2% and 1% of the IgG and IgA levels in serum, respectively. We found a correlation between the HPV16 and 18 antibody levels in serum and CVS up to two years after the first vaccination indicating that probably transudation and/or exudation of antibodies from the systemic circulation to the cervical mucosa takes place.

In this study, CVS was self-sampled with a tampon while in other studies CVS is mostly collected with sponges,[8,9,11] which have to be placed in the cervix by a physician. This might be unpleasant, and is not suitable for large-scale studies. The girls found the tampon as collection method easy in use and comfortable. This collection method might contribute to better participation rates in large-scale studies. In addition, the determination of HPV-specific antibodies in CVS was also easy after extraction of the sample and the tampon collection method had no effect on the antibody concentrations.

The menstrual cycle has been reported to have effect on antibody levels in the genital tract.[12] Around ovulation Ig antibody levels might decrease because of a protective mechanism that reduces the level of anti-sperm antibodies in the female genital tract at the time of ovulation.[13] Kemp et al. found differences between IgG levels in serum and CVS during the menstrual cycle based on a small number of observations.[8] Therefore, information was collected on the last menstruation from the participants. We relied on self-reported data that might be less accurate than detection of sex hormones during the menstrual cycle.[13] We did

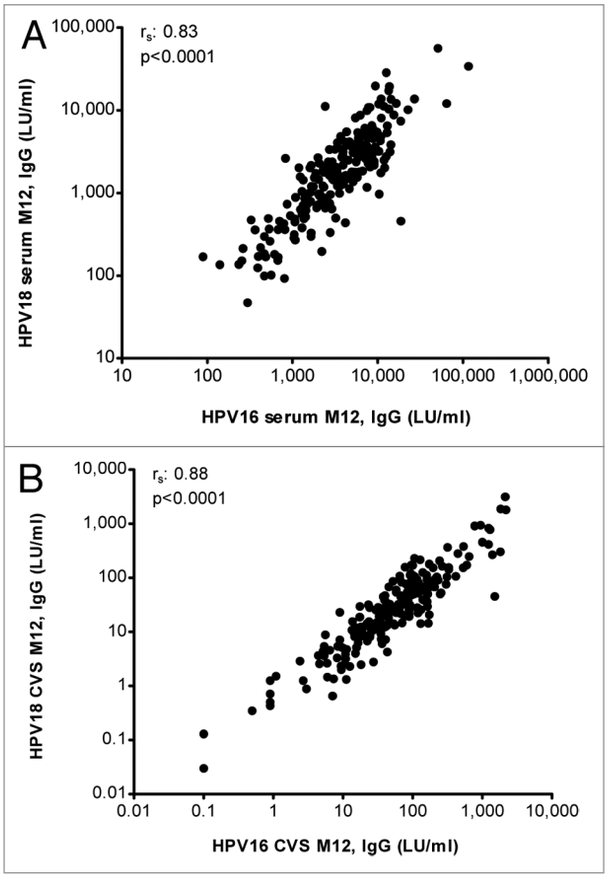

Figure 6. Spearman rank correlations (r_s) between IgG antibody levels of HPV16 and HPV18 in serum (**A**) and cervical secretion samples (CVS) (**B**) one year after the first vaccination (M12). X and Y-axis are in logarithmic scale.

not find an effect between non-OC and OC users even when we normalized to the total amount of IgG.

The increase in HPV IgG seroprevalence in non-vaccinated individuals is similar to the step-up in HPV16 seroprevalence as observed in a large Dutch sero-epidemiological study in young adolescent girls.[14] IgG antibodies in CVS were very low and near detection limits indicating that antibody measurements in CVS in a non-vaccinated population might not be suitable for the detection of naturally derived HPV-specific antibodies.

To prevent HPV from entering the keratinocytes in the cervix, prophylactic HPV vaccination should induce a first line of defense by eliciting sufficient amounts of antibodies.[15] We found detectable antibody levels in CVS among vaccinated girls but these levels were approximately 60-fold lower than serum levels. Antibody levels in CVS remained constant for at least two years post-vaccination. In 10% and 30% of the girls, we were not able to detect HPV16 and 18 antibody levels in CVS, respectively, although HPV16 and 18 antibody levels in serum were high. These findings were comparable with other studies[1,8,9,16] although in most studies less stringent exclusion criteria were handled for blood contamination in CVS as compared with our study. Despite a high vaccine efficacy against precancerous cervical lesions associated with HPV16/18 or cross-reactive HPV types,[3,6,17] girls without detectable antibodies in CVS might be the first at risk for HPV infections. However, a recent study showed that in mice also low vaccine-derived antibody concentrations can neutralize the virus and inhibit the binding of the virus to the basal cells of the cervical epithelium at another stage of infection.[18,19] Although we showed that after HPV16/18 vaccination cross-reactive antibodies against HPV52 and 58 were detectable in both serum and CVS, the biological relevance of these antibodies is probably limited, as vaccine monitoring studies did not observe any cross-protection.[5,6]

Moreover, at present the lowest level at which HPV16 and 18 antibodies exert their protective effect against an HPV infection is unknown.

Although in most mucosal tissues IgA-producing cells are predominant, the endocervical mucosa contains a higher proportion of plasma-derived or locally produced IgG antibodies, which are detectable in cervico-vaginal secretions.[13,20-22] The higher IgG proportions in cervico-vaginal secretions might also depend on the location of sampling and different sampling techniques.[21] However, after natural infection it is shown that HPV specific IgA antibodies are detectable at the cervix, mainly in persistent HPV infections.[23-26] Data about IgA antibody responses after vaccination are limited. Although IgA levels were much lower as compared with IgG levels, we detected HPV16 and 18 specific IgA responses with high correlations between serum and CVS. Whether these vaccine-derived HPV16 and 18 IgA antibody levels in the systemic circulation play a role in HPV neutralization is unknown. However, Bontkes et al. already observed that after HPV infections systemic IgA responses in women with abnormal cytology were actually more associated with HPV clearance than locally produced IgA at the cervical mucosa. The systemic IgA responses might be an indication of a successful cellular immune response induced at the local lymph nodes, mediated by cytokines.[27]

The correlations of HPV16 and 18 IgG and IgA antibody levels between serum and CVS might denote that vaccine derived antibodies transudate from the systemic circulation to the cervical mucosa.[15,28] As antibody levels in CVS remained constant for at least 2 y post-vaccination, these levels might contribute to a protective environment at the cervix. This hypothesis is supported by the high correlations between antibody levels specific for diphtheria and tetanus toxoid in serum and CVS because these vaccine-induced antibodies are not produced at the cervical matrix. In CVS, increasing HPV antibody concentrations were found when more erythrocytes were detected. This indicates that exudated blood probably originating from small lesions in the cervix also can contribute to the presence of HPV specific antibodies at the cervix. Protection at the cervix is not necessarily only based on antibodies that transudate or exudate from the systemic circulation to the mucosa. It can also be facilitated by other immune mechanisms e.g., local production of antibodies in which IgG and IgA are produced primarily in mucosal associated lymphoid tissues and are actively transported into mucosal secretions.[29]

In conclusion, after vaccination with the bivalent HPV vaccine HPV16 and 18 IgG and IgA antibodies were detectable in

CVS and these antibody concentrations correlate well with serum antibody levels. Antibody levels in CVS were lower as compared with serum and levels remained constant up to two years post-vaccination. The correlation between Tt and Dt IgG antibodies in serum and CVS suggests that vaccine induced antibodies in the systemic circulation might transudate and/or exudate to the cervical mucosa although other immune mechanisms cannot be excluded. These important immune mechanisms probably contribute to sufficient antibody levels at sites where HPV infections actually take place and therefore can provide protection against HPV infection and/or re-infections.

Materials and Methods

Study design. A random sample of 9,500 girls aged 14–16 y eligible for the national catch-up HPV vaccinations was invited into the HAVANA (HPV Among Vaccinated And Non-vaccinated Adolescents) study of which 1151 (12%) girls participated in the study.[30] Each girl was asked to fill in a questionnaire, to provide a blood sample and optionally a CVS by using a tampon. Both CVS and blood samples were available from 737 girls pre-vaccination (M0, 2009), from 451 girls one year after the first vaccination (M12, 2010), and from 459 girls two years after the first vaccination (M24, 2011). A signed informed consent was obtained from all participants and their parents. The study was approved by the medical ethics review committee of the Free University Amsterdam (approval number: 2009/22).

Collection of cervical secretion and serum samples. Cervical secretion was collected using a tampon (mini-pro comfort, OB, Johnson and Johnson Consumer). Girls were asked to use a tampon for 2 h when they were not menstruating. CVS were collected from the tampons by addition of 0.5 ml of PBS containing complete protease inhibitor cocktail (1 tablet/50 ml, Roche Diagnostics catalog No. 11836145001) and subsequent centrifugation for 30 min, 3,200 g at 4°C. To assess for the presence of blood, CVS were evaluated using the Hemastix® (Siemens Healthcare Diagnostics Inc., catalog No. 2597428) reagent strip test according to manufactures instructions. CVS were categorized into the following categories: no trace, 20, 25, 80, 200 and > 200 erythrocytes/μl.

VLP-based multiplex immunoassay. CVS and serum samples were stored at -80°C until analysis. For the measurement of HPV-specific IgG and IgA antibodies against HPV L1 virus-like-particles (VLP) 16, 18, 31, 33, 45, 52 and 58, a VLP-based multiplex immunoassay (MIA) was used as earlier described.[14] GSK (GlaxoSmithKline Biologicals S.A., Rixensart, Belgium) kindly supplied the HPV-VLPs. Briefly, VLPs were coupled to fluorescent microspheres. Sera and CVS were incubated with the microspheres in a 1/50 and 1/100 dilution (pre-vaccination) and in a 1/50 up to 10,000 dilution (post-vaccination). HPV-specific IgG and IgA antibodies were detected using a 1/200 dilution of R-phycoerythrin conjugated goat anti-human IgG or IgA (Jackson ImmunoResearch laboratories Inc., catalog No. 109–116–098) in PBS. Four in-house control sera and an in-house standard were used on each plate. The in-house standard (IVIG, lot LE12H227AF, Baxter catalog No. 1500912) was calibrated against reference serum of GSK for all the seven HPV types. HPV specific antibodies were analyzed using the Bioplex system 200 with Bioplex software (Bio-Rad Laboratories). Sera were assumed to be IgG seropositive at the following cut-offs determined previously with this assay: 9, 13, 27, 11, 19, 14 and 31 Luminex Units/ml (LU/ml) for HPV16, 18, 31, 33, 45, 52 and 58, respectively.

As no cut-off values for CVS are known, we used the serum cut-off values as an arbitrary cut-off for IgG seropositivity in CVS. IgA antibody concentrations were expressed in arbitrary RIVM U/ml using the in-house standard as reference serum. No international HPV-specific IgA reference serum is available and cut-off values for IgA seropositivity could not be determined. To test for possible inter-immunoglobulin isotype competition, a panel of serum samples (n = 40) were depleted of IgG by adding GullSORB (10:1 vol/vol) (Meridian Bioscience Inc., catalog No. XX715). We did not observe any interference of IgG in the IgA measurement.

IgG antibodies against tetanus and diphtheria toxoid in serum and CVS were measured using a multiplex immunoassay as described previously.[31,32] In order to account for the variation of the IgG levels in CVS during the menstrual cycle, total IgG was measured in a subset of CVS and serum samples (n = 35).

Statistical methods. All analyses were performed in GraphPad Prism version 5. For the analyses, results were included from girls who had been vaccinated three times with the bivalent HPV vaccine, who delivered both a CVS and serum sample at M0, M12 and/or M24 and from whom the CVS contained a blood trace of ≤ 25 erythrocytes/μl. Significant ($p < 0.05$) differences in GMCs were calculated using a Mann-Whitney test and differences between paired samples were calculated with the paired t-test of log-transformed data. Correlations between antibody concentrations in CVS and serum were calculated using the Spearman rank correlation coefficient (r_s).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Schwarz TF, Kocken M, Petäjä T, Einstein MH, Spaczynski M, Louwers JA, et al. Correlation between levels of human papillomavirus (HPV)-16 and 18 antibodies in serum and cervicovaginal secretions in girls and women vaccinated with the HPV-16/18 AS04-adjuvanted vaccine. Hum Vaccin 2010; 6:1054-61; PMID:21157180; http://dx.doi.org/10.4161/hv.6.12.13399.
2. Stanley M. Immunobiology of HPV and HPV vaccines. Gynecol Oncol 2008; 109(Suppl):S15-21; PMID:18474288; http://dx.doi.org/10.1016/j.ygyno.2008.02.003.
3. Lehtinen M, Paavonen J, Wheeler CM, Jaisamrarn U, Garland SM, Castellsagué X, et al.; HPV PATRICIA Study Group. Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. Lancet Oncol 2012; 13:89-99; PMID:22075171.
4. Muñoz N, Manalastas R Jr., Pitisuttithum P, Tresukosol D, Monsonego J, Ault K, et al. Safety, immunogenicity, and efficacy of quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine in women aged 24-45 years: a randomised, double-blind trial. Lancet 2009; 373:1949-57; PMID:19493565; http://dx.doi.org/10.1016/S0140-6736(09)60691-7.
5. Kemp TJ, Hildesheim A, Safaeian M, Dauner JG, Pan Y, Porras C, et al. HPV16/18 L1 VLP vaccine induces cross-neutralizing antibodies that may mediate cross-protection. Vaccine 2011; 29:2011-4; PMID:21241731; http://dx.doi.org/10.1016/j.vaccine.2011.01.001.
6. Wheeler CM, Castellsagué X, Garland SM, Szarewski A, Paavonen J, Naud P, et al.; HPV PATRICIA Study Group. Cross-protective efficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. Lancet Oncol 2012; 13:100-10; PMID:22075170.
7. De Carvalho N, Teixeira J, Roteli-Martins CM, Naud P, De Borba P, Zahaf T, et al. Sustained efficacy and immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine up to 7.3 years in young adult women. Vaccine 2010; 28:6247-55; PMID:20643092; http://dx.doi.org/10.1016/j.vaccine.2010.07.007.
8. Kemp TJ, García-Piñeres A, Falk RT, Poncelet S, Dessy F, Giannini SL, et al.; Costa Rica Vaccine Trial (CVT) Group. Evaluation of systemic and mucosal anti-HPV16 and anti-HPV18 antibody responses from vaccinated women. Vaccine 2008; 26:3608-16; PMID:18541349; http://dx.doi.org/10.1016/j.vaccine.2008.04.074.
9. Petäjä T, Pedersen C, Poder A, Strauss G, Catteau G, Thomas F, et al. Long-term persistence of systemic and mucosal immune response to HPV-16/18 AS04-adjuvanted vaccine in preteen/adolescent girls and young women. Int J Cancer 2011; 129:2147-57; PMID:21190190.
10. Rondy M, van Lier A, van de Kassteele J, Rust L, de Melker H. Determinants for HPV vaccine uptake in the Netherlands: A multilevel study. Vaccine 2010; 28:2070-5; PMID:20045095; http://dx.doi.org/10.1016/j.vaccine.2009.12.042.
11. Kemp TJ, Hildesheim A, Falk RT, Schiller JT, Lowy DR, Rodriguez AC, et al. Evaluation of two types of sponges used to collect cervical secretions and assessment of antibody extraction protocols for recovery of neutralizing anti-human papillomavirus type 16 antibodies. Clin Vaccine Immunol 2008; 15:60-4; PMID:18032596; http://dx.doi.org/10.1128/CVI.00118-07.
12. Safaeian M, Falk RT, Rodriguez AC, Hildesheim A, Kemp T, Williams M, et al. Factors associated with fluctuations in IgA and IgG levels at the cervix during the menstrual cycle. J Infect Dis 2009; 199:455-63; PMID:19133811; http://dx.doi.org/10.1086/596060.
13. Nardelli-Haefliger D, Wirthner D, Schiller JT, Lowy DR, Hildesheim A, Ponci F, et al. Specific antibody levels at the cervix during the menstrual cycle of women vaccinated with human papillomavirus 16 virus-like particles. J Natl Cancer Inst 2003; 95:1128-37; PMID:12902442; http://dx.doi.org/10.1093/jnci/djg018.
14. Scherpenisse M, Mollers M, Schepp RM, Boot HJ, de Melker HE, Meijer CJ, et al. Seroprevalence of seven high-risk HPV types in The Netherlands. Vaccine 2012; 30:6686-93; PMID:22959981; http://dx.doi.org/10.1016/j.vaccine.2012.08.068.
15. Schwarz TF, Leo O. Immune response to human papillomavirus after prophylactic vaccination with AS04-adjuvanted HPV-16/18 vaccine: improving upon nature. Gynecol Oncol 2008; 110(Suppl 1):S1-10; PMID:18653222; http://dx.doi.org/10.1016/j.ygyno.2008.05.036.
16. Einstein MH, Baron M, Levin MJ, Chatterjee A, Edwards RP, Zepp F, et al.; HPV-010 Study Group. Comparison of the immunogenicity and safety of Cervarix and Gardasil human papillomavirus (HPV) cervical cancer vaccines in healthy women aged 18-45 years. Hum Vaccin 2009; 5:705-19; PMID:19684472; http://dx.doi.org/10.4161/hv.5.10.9518.
17. Jenkins D. A review of cross-protection against oncogenic HPV by an HPV-16/18 AS04-adjuvanted cervical cancer vaccine: importance of virological and clinical endpoints and implications for mass vaccination in cervical cancer prevention. Gynecol Oncol 2008; 110(Suppl 1):S18-25; PMID:18653221; http://dx.doi.org/10.1016/j.ygyno.2008.06.027.
18. Day PM, Kines RC, Thompson CD, Jagu S, Roden RB, Lowy DR, et al. In vivo mechanisms of vaccine-induced protection against HPV infection. Cell Host Microbe 2010; 8:260-70; PMID:20833377; http://dx.doi.org/10.1016/j.chom.2010.08.003.
19. Longet S, Schiller JT, Bobst M, Jichlinski P, Nardelli-Haefliger D. A murine genital-challenge model is a sensitive measure of protective antibodies against human papillomavirus infection. J Virol 2011; 85:13253-9; PMID:21976653; http://dx.doi.org/10.1128/JVI.06093-11.
20. Mestecky J, Moldoveanu Z, Russell MW. Immunologic uniqueness of the genital tract: challenge for vaccine development. Am J Reprod Immunol 2005; 53:208-14; PMID:15833098; http://dx.doi.org/10.1111/j.1600-0897.2005.00267.x.
21. Brandtzaeg P. Mucosal immunity in the female genital tract. J Reprod Immunol 1997; 36:23-50; PMID:9430737; http://dx.doi.org/10.1016/S0165-0378(97)00061-2.
22. Mestecky J, Fultz PN. Mucosal immune system of the human genital tract. J Infect Dis 1999; 179(Suppl 3):S470-4; PMID:10099122; http://dx.doi.org/10.1086/314806.
23. Monroy OL, Aguilar C, Lizano M, Cruz-Talonia F, Cruz RM, Rocha-Zavaleta L. Prevalence of human papillomavirus genotypes, and mucosal IgA anti-viral responses in women with cervical ectopy. J Clin Virol 2010; 47:43-8; PMID:19906557; http://dx.doi.org/10.1016/j.jcv.2009.10.008.
24. Palmroth J, Namujju P, Simen-Kapeu A, Kataja V, Surcel HM, Tuppurainen M, et al. Natural seroconversion to high-risk human papillomaviruses (hrHPVs) is not protective against related HPV genotypes. Scand J Infect Dis 2010; 42:379-84; PMID:20121650; http://dx.doi.org/10.3109/00365540903501608.
25. Sasagawa T, Rose RC, Azar KK, Sakai A, Inoue M. Mucosal immunoglobulin-A and -G responses to oncogenic human papilloma virus capsids. Int J Cancer 2003; 104:328-35; PMID:12569556; http://dx.doi.org/10.1002/ijc.10939.
26. Bard E, Riethmuller D, Meillet D, Prétet JL, Schaal JP, Mougin C, et al. High-risk papillomavirus infection is associated with altered antibody responses in genital tract: non-specific responses in HPV infection. Viral Immunol 2004; 17:381-9; PMID:15357904; http://dx.doi.org/10.1089/vim.2004.17.381.
27. Bontkes HJ, de Gruijl TD, Walboomers JM, Schiller JT, Dillner J, Helmerhorst TJ, et al. Immune responses against human papillomavirus (HPV) type 16 virus-like particles in a cohort study of women with cervical intraepithelial neoplasia. II. Systemic but not local IgA responses correlate with clearance of HPV-16. J Gen Virol 1999; 80:409-17; PMID:10073701.
28. Barr E, Koutsky LA. Re: Specific antibody levels at the cervix during the menstrual cycle of women vaccinated with human papillomavirus 16 virus-like particles. J Natl Cancer Inst 2004; 96:412-3, author reply 413-4; PMID:14996866; http://dx.doi.org/10.1093/jnci/djh071.
29. Naz RK. Female genital tract immunity: distinct immunological challenges for vaccine development. J Reprod Immunol 2012; 93:1-8; PMID:22154945; http://dx.doi.org/10.1016/j.jri.2011.09.005.
30. Mollers M, Scherpenisse M, van der Klis FR, King AJ, van Rossum TG, van Logchem EM, et al. Prevalence of genital HPV infections and HPV serology in adolescent girls, prior to vaccination. Cancer Epidemiol 2012.
31. van Gageldonk PG, van Schaijk FG, van der Klis FR, Berbers GA. Development and validation of a multiplex immunoassay for the simultaneous determination of serum antibodies to Bordetella pertussis, diphtheria and tetanus. J Immunol Methods 2008; 335:79-89; PMID:18407287; http://dx.doi.org/10.1016/j.jim.2008.02.018.
32. van Gageldonk PG, von Hunolstein C, van der Klis FR, Berbers GA. Improved specificity of a multiplex immunoassay for quantitation of anti-diphtheria toxin antibodies with the use of diphtheria toxoid. Clin Vaccine Immunol 2011; 18:1183-6; PMID:21613460; http://dx.doi.org/10.1128/CVI.05081-11.